



Distinct colony and persister resistant populations develop in response to vertical MAPK inhibition in a model of KRAS-mutant colorectal cancer

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BACKGROUND & OBJECTIVES

- Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the US.¹
- KRAS mutation is present in 45% of all CRC cases¹, but no targeted therapies have been approved.
- Vertical MAPK inhibition therapy consisting of MEK and CDK4/6 inhibitors is currently in a Phase II clinical trial.
- Despite the initial response to targeted therapies, most cancers develop resistance.
- The aims of this project include:
 - Modeling resistance of KRAS-mutated CRC to vertical MAPK inhibitor combination therapies in vitro.
 - Investigating the mechanisms driving resistance.

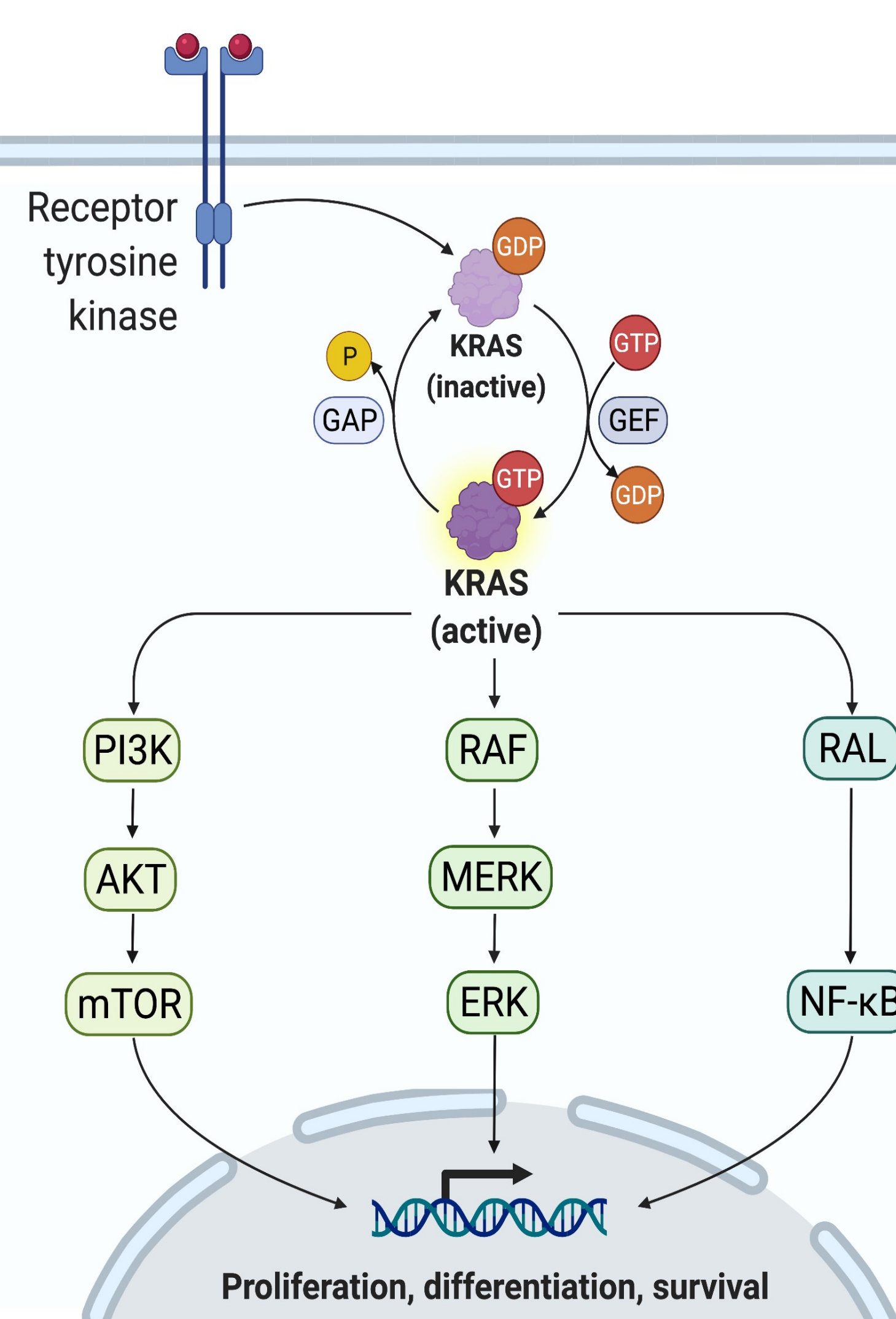


Figure 1. Overview of the KRAS pathway.

METHODS

1. Developing Resistance

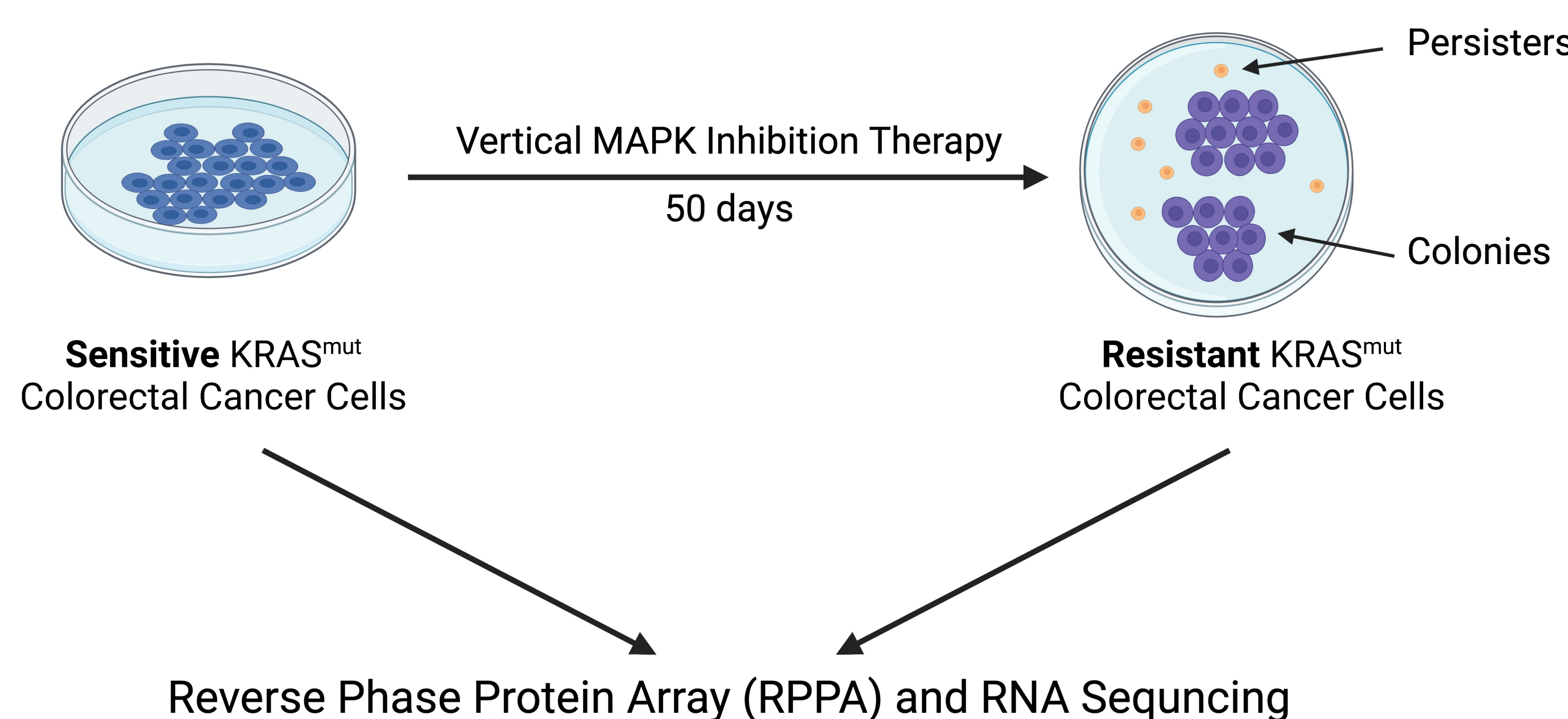


Figure 2. KRAS^{mut} colorectal cancer cells were drugged with vertical MAPK inhibition combination therapy to develop resistance. Two distinct resistant phenotypes identified: colonies and persister cells. RNA-seq analysis was conducted on both sensitive and resistant cells to identify differentially expressed genes.

2. Gateway Cloning

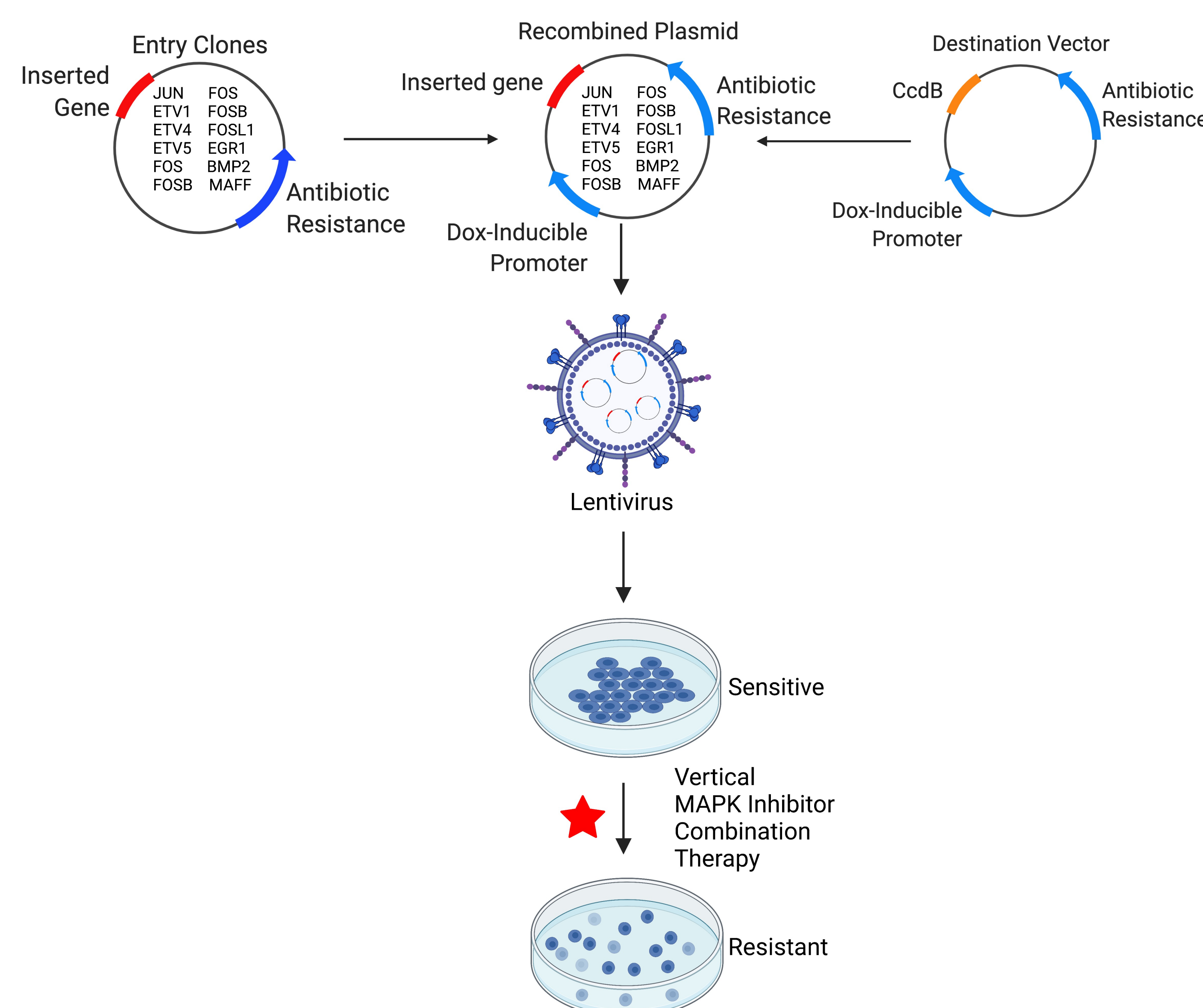


Figure 3. Fifteen (15) entry clones corresponding to the 15 differentially expressed genes were designed and introduced into a doxycycline-inducible destination vector. The plasmids were then transduced into KRAS^{mut} CRC cells using lentiviruses.

RESULTS

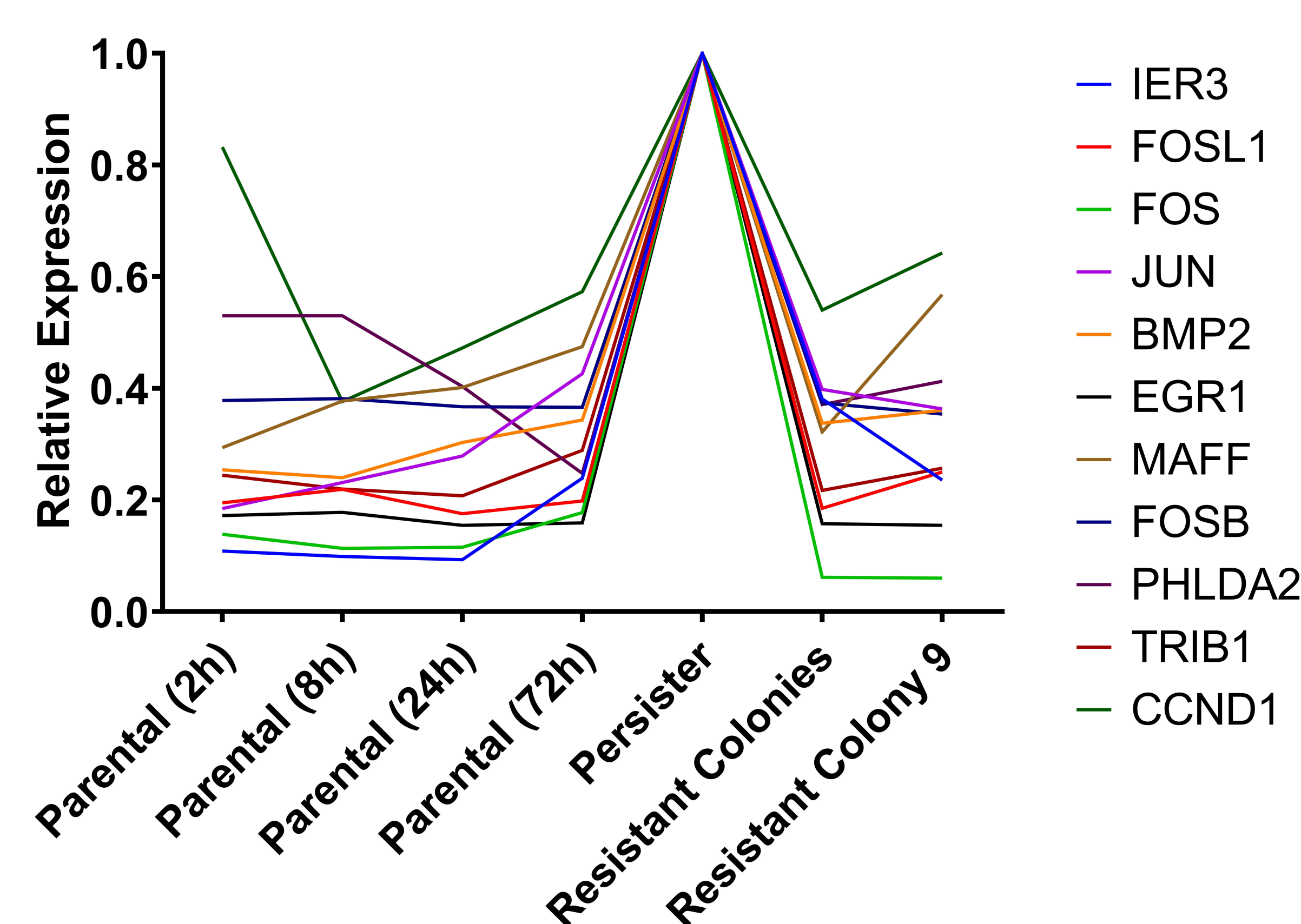


Figure 4. RNA-seq identified eleven (11) genes that are upregulated in persisters, compared to parental cells and resistant colonies. The genes are implicated in the MAPK pathway.

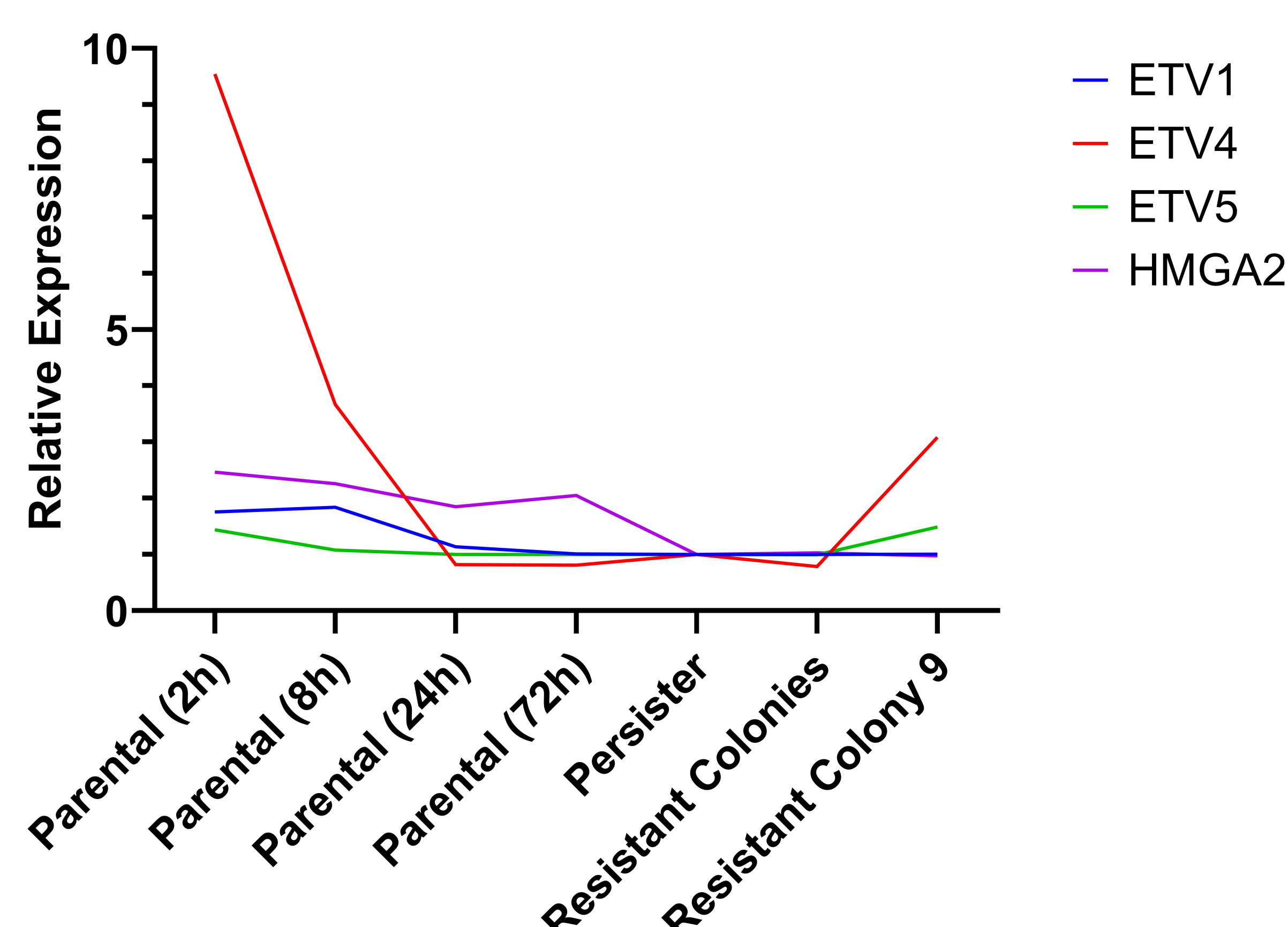


Figure 5. RNA-seq identified four (4) genes that are differentially expressed across the different cell types. The genes are implicated in the MAPK pathway.

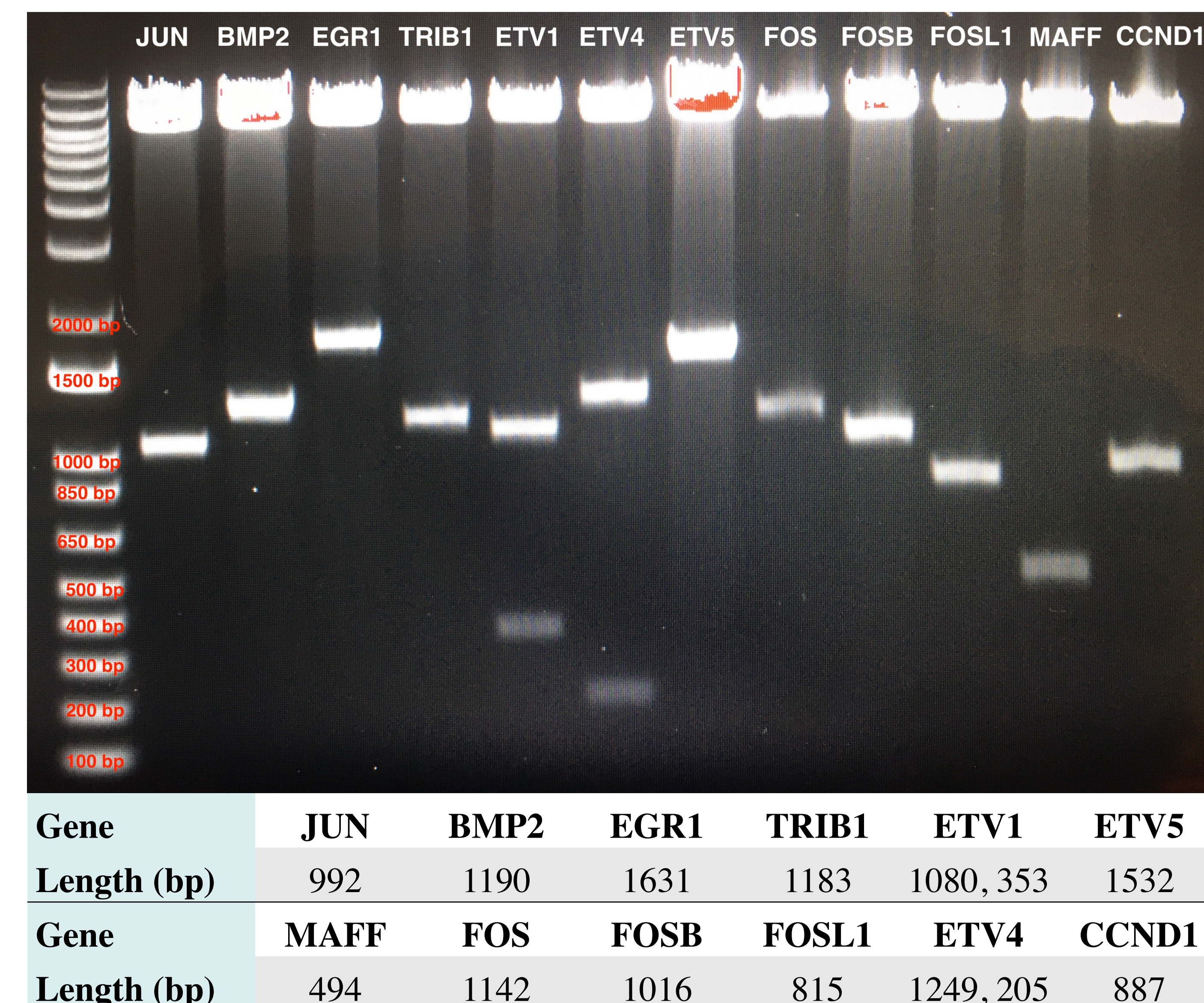


Figure 6. Gel electrophoresis confirming the formation of the correct Gateway cloning plasmids for 12 target genes. The plasmids had been digested with the BsrGI restriction enzyme.

DISCUSSION

We determined that:

- KRAS^{mut} colorectal cancer cells acquire resistance to vertical MAPK inhibition combination therapy upon long-term exposure.
- Resistant cells display two distinct phenotypes: isolated persisters and resistant colonies.
- Eleven (11) genes are upregulated in persisters and four (4) are differentially expressed across all cell types.

We successfully designed plasmids that will enable us to overexpress each of the 15 genes in KRAS^{mut} colorectal cancer cells.

Future directions:

- Overexpress the genes in parental cells prior to drugging to determine whether they confer resistance.
- Overexpress the genes in resistant colonies to determine whether they produce the persister phenotype.

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- Figures made using BioRender and Prism.